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J. Parasitol. 30:295-302, 1944.

J. Protozool., 8:410-416, 1961.

J. Parasitol. 73:311-326, 1976.

Poultry Sci. 54:2081-2086, 1975.

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Wight, P. A. L., 1963. Lymphoid leukosis and fowl paralysis in the quail. Vet. Rec. 75: 685-687.

A Rapid Technique for the Cleaning and Concentration of *Eimeria* Oocysts

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ABSTRACT A technique using 1% sodium bicarbonate, ether and centrifugation was developed for cleaning oocysts of *Eimeria tenella*, *E. maxima* or *E. mivati*. The speed and efficiency of this technique was compared with the sedimentation technique currently used in many laboratories. Parameters measured were percent sporulation after cleaning and number of oocysts recovered. Suspensions of oocysts cleaned by bicarbonate and ether consistently yielded a similar or superior percent sporulation. In four of the five trials bicarbonate and ether recovered a larger number of oocysts from feces.

To determine the storage stability of oocysts cleaned with bicarbonate and ether in comparison with sedimentation cleaned oocysts, suspensions of *Eimeria tenella* and *E. mivati* were stored at 4° C. and assayed initially after harvest and at three month intervals thereafter. The parameters of bird weight gain, lesion score, mortality and feed conversion were measured to test viability of oocysts. Bicarbonate and ether cleaning had no initial deleterious effect on oocyst viability. After 6 months storage, oocysts of *Eimeria* cleaned with bicarbonate and ether maintained viability similar or superior to oocysts cleaned by sedimentation.

POULTRY SCIENCE 54: 2081-2086, 1975

INTRODUCTION

THE study of coccidiosis in the laboratory often requires that large numbers of oocysts be harvested and stored with minimal loss of virulence. Previous techniques have dealt primarily with the quantitative retrieval of small numbers of oocysts for determining the severity of infection (Farr and Luttermoser, 1951; Beach, 1943; Gill, 1954; Dorney, 1964; Ishii and Ohnaga, 1970) or were designed to clean small amounts of fecal material (Sharma *et al.*, 1963; Wagenbach *et al.*, 1966).

Vetterling (1969) described a differential density flotation method using sucrose gradients and continuous-flow chemical centrifugation. This technique is effective for separating oocysts from large quantities of fecal debris, and requires a technician's time of one man-hour to clean one liter of fecal suspension. However, in surveying previous techniques, the use of sucrose limited storage viability to only a few weeks (Reid, personal communication).

Edgar (1964) described a simple technique for separating oocysts from large quantities of fecal material. By allowing the heavier particles to sediment from a suspension of fecal material, the lighter oocysts could be collected along with the supernatant. This technique, however, generates large volumes of waste water and the extensive washing to free oocysts from debris results in a progressive loss of oocysts. Also, to achieve maximum storage viability, extensive washing necessitates a waiting time of upwards of 12 hours.

The objective of this study was to develop a technique which would rapidly separate

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oocysts from fecal material with only a minimal loss during the process, and which would allow satisfactory storage of oocysts.

MATERIALS AND METHODS

Feces were collected from chickens previously inoculated with *Eimeria tenella*, *E. maxima*, and *E. mivati*. The fecal material was strained through cheese cloth into a large beaker with several washings of water.

An aliquot of this strained fecal suspension was removed and cleaned and concentrated by the "bicarbonate-ether" technique as follows:

1) The suspension was added to 50 ml. centrifuge tubes, spun for 5 minutes and the supernatant discarded.
2) The sediment was resuspended in 35 ml. of 1% sodium bicarbonate.
3) A 3-5 ml. layer of ether was added and the contents mixed by vigorous shaking.
4) This mixture was centrifuged for 5 minutes and the debris plug and supernatant discarded.
5) The sediment was washed by resuspension in water, centrifuged for 5 minutes and the supernatant discarded.

For comparison with the bicarbonate-ether technique, a modification of the sedimentation technique (Edgar, 1964) currently used in many laboratories was chosen. The remaining aliquot of the original fecal suspension was thoroughly mixed and allowed to stand. When a ring of denser debris formed near the bottom of the beaker, the supernatant was decanted off and saved. This supernatant contained oocysts as well as smaller fecal particles. To extract larger quantities of oocysts from the sediment, repeated resuspension and settling of the sediment was performed. Each supernatant was saved and either centrifuged in 500 ml. bottles for 5 minutes or allowed to settle overnight. The supernatant after centrifugation was discarded. All centrifugations were performed at a slow speed to avoid destroying oocysts.

Sediments collected were pooled according to cleaning technique, suspended in 2% potassium dichromate and aerated for 115 hours at room temperature. The oocysts in each of these solutions were then counted in a Neubauer haemocytometer and classed as sporulated (containing distinct sporocysts) or unsporulated. Based on these counts, calculations were made to estimate the number of oocysts in the original fecal suspension.[2] The solutions were stored tightly stoppered in 2% potassium dichromate at 4° C. until further use.

The stock solutions from each technique were assayed for viability initially within two weeks of harvest and thereafter at three month intervals. White Leghorn cockerels were inoculated orally with 50,000, 100,000, or 200,000 sporulated oocysts per bird. Ten birds were inoculated at each dose and a seventh group was used as an uninoculated control (Gardiner and Wehr, 1950). Each assay was terminated 7 days after inoculation. Parameters used to measure viability were those employed in coccidiostat evaluation: 1) individual weight gain, 2) lesion score (Johnson and Reid, 1970), 3) feed conversion (total amount of feed consumed by a treatment group ÷ total weight gain of the treatment group), and 4) mortality.

Differences between means of weight gain and lesion score were tested for significance with the Duncan multiple range test (Duncan, 1955). Main effects and interactions between doses and the two concentration techniques were tested for significance with analysis of

2. $N = n \cdot s \cdot V_1 / V_2$, where "N" is the estimated number of oocysts in the original fecal suspension, "n" is the number of oocysts per milliliter of stock solution, "s" is the volume of the stock solution, "V_1" is the volume of the original fecal suspension, and "V_2" is the volume of the aliquot before cleaning and concentration.

variance. The use of the word 'significant' in this paper has a statistical connotation ($P \leq 0.05$).

RESULTS AND DISCUSSION

When time is not a limiting factor, Edgar's (1964) method is efficient for separating oocysts from large quantities of fecal material with the oocysts collected maintaining excellent storage viability. The various salt or sugar flotation techniques are rapid for cleaning oocysts from fecal debris, but are not capable of cleaning large quantities of fecal material and the oocysts collected maintain their viability for only a few weeks. A method is needed which combines the ability to clean large quantities of fecal material rapidly and efficiently when oocysts are needed the same day as the feces are collected while maintaining the storage stability of the oocysts. Our method satisfies these criteria.

Decreasing the fecal content in an oocyst suspension increases the number of oocysts that will sporulate (Edgar, personal communication). The percent sporulation of oocysts after cleaning with bicarbonate-ether was in every trial equal or superior to sporulation after sedimentation cleaning (Table 1). Percent sporulation of bicarbonate-ether-cleaned *E. maxima* oocysts was consistently superior to *E. maxima* oocysts cleaned by sedimentation. The oocysts of this species are the largest infecting chickens and in solution settle rapidly. To insure recovery of sufficient numbers of oocysts during sedimentation, decanting of the supernatant must be accomplished before the *E. maxima* oocysts settle. This also involves retrieval of corresponding large quantities of debris which are in suspension and impair sporulation. Bicarbonate-ether cleans fecal debris from oocyst suspensions to a degree which enhances *E. maxima* sporulation. The difference of percent sporulation of *E. tenella*, trial 1, is probably due to unnecessary recovery of large amounts of debris during decanting of oocysts. This problem was corrected in *E. tenella*, trial 2.

Recovery of the maximum number of oocysts from fecal material is important in that it provides a stock solution containing sufficient infective oocysts for coccidiosis studies. With the exception of *E. tenella*, trial 2, bicarbonate-ether consistently recovered statistically superior numbers of oocysts (Table 1). Since the entire aliquot was directly cleaned by the bicarbonate-ether technique, theoretically, the recovery of oocysts should be near the maximum. This is in contrast to the sedimentation technique in which oocysts are lost along with fecal debris during the alternate settling and decanting of the aliquot.

It was possible that the sodium bicarbonate or the ether used in this technique might limit the viability of stored oocysts. To determine

TABLE 1.—*The effectiveness of the bicarbonate-ether and sedimentation methods in cleaning and concentrating oocysts of* Eimeria *from feces*[1]

Species	Trial	Percent sporulation[2]		Number of oocysts (in millions)[3]	
		Bicarbonate-ether	Sedimentation	Bicarbonate-ether	Sedimentation
E. tenella	1	84.07 ± 1.21b	72.35 ± 1.21a	164.8 ± 8.9*	82.4 ± 0.9
E. tenella	2	82.35 ± 1.35b	82.64 ± 1.36b	260.0 ± 10.5	350.5 ± 11.9*
E. maxima	1	30.71 ± 1.44b	19.26 ± 0.32a	69.5 ± 6.4*	41.8 ± 1.9
E. maxima	2	32.15 ± 1.91b	21.59 ± 0.69a	28.0 ± 1.8*	13.9 ± 2.1
E. mivati	1	51.63 ± 0.58a	49.79 ± 0.57a	265.1 ± 4.8*	240.6 ± 7.9

[1] Each value is the mean of 5 replicates.
[2] Within a species, values followed by a common letter are not significantly different.
[3] *Designates a significant difference between methods within a species replicate ($P \leq 0.05$).

TABLE 2.—*Initial and three-month assays of* Eimeria mivati *comparing efficacy of bicarbonate-ether and sedimentation methods of cleaning oocysts*[1,2]

Parameter	Dose	Initial assay		Three-month assay	
		Bicarbonate-ether	Sedimentation	Bicarbonate-ether	Sedimentation
Weight gain (gm.)[3]	Control	82.1 ± 3.6a		106.4 ± 4.6a	
	50,000	51.3 ± 5.2b	47.7 ± 5.7bc	52.0 ± 3.9b	62.9 ± 2.5b
	100,000	39.8 ± 6.7bcd	40.6 ± 3.6bcd	32.8 ± 5.6cd	50.5 ± 4.6b
	200,000	21.8 ± 11.8d	30.3 ± 3.9cd	15.6 ± 6.8d	24.5 ± 5.3cd
Lesion score[3]	Control	0.0a		0.0a	
	50,000	2.3 ± 0.2b	2.1 ± 0.2b	2.6 ± 0.2c	2.0 ± 0.2b
	100,000	3.2 ± 0.1c	3.2 ± 0.2c	3.4 ± 0.3d	2.6 ± 0.2c
	200,000	3.1 ± 0.2c	3.4 ± 0.2c	3.5 ± 0.2d	3.4 ± 0.2d
Feed conversion	Control	2.192		2.488	
	50,000	2.959	3.306	3.700	3.452
	100,000	4.300	4.318	5.399	4.311
	200,000	5.532	5.208	9.904	8.380

[1]Within each parameter of each assay, values followed by a common letter are not significantly different ($P \leq 0.05$).
[2]No mortality was recorded for any assay, method or dose.
[3]Each value is the mean of 10 replicates.

TABLE 3.—*Initial, three-month and six-month assays of* Eimeria tenella *(trial 1) comparing efficacy of bicarbonate-ether and sedimentation methods of cleaning oocysts*[1]

Parameter	Dose	Initial assay		Three-month assay		Six-month assay	
		Bicarbonate-ether	Sedimentation	Bicarbonate-ether	Sedimentation	Bicarbonate-ether	Sedimentation
Mortality (%)	Control	0		0		0	
	50,000	40	30	20	30	0	10
	100,000	40	50	30	40	20	0
	200,000	70	50	20	30	0	0
Weight gain (gm.)[2]	Control	73.5 ± 4.0a		84.7 ± 4.3a		96.4 ± 5.2a	
	50,000	32.6 ± 4.1bc	29.8 ± 4.7bc	32.9 ± 4.2bc	40.0 ± 4.5b	66.6 ± 6.0b	75.9 ± 8.3ab
	100,000	23.9 ± 5.5cd	22.1 ± 4.4cde	25.6 ± 8.5bcd	33.4 ± 4.6bc	39.7 ± 10.9c	65.5 ± 8.3b
	200,000	16.0 ± 3.0de	9.9 ± 2.4e	14.9 ± 6.0d	19.3 ± 6.4cd	7.9 ± 10.4e	25.7 ± 6.0cd
Lesion score[2]	Control	0.0a		0.0a		0.0a	
	50,000	3.4 ± 0.3b	3.5 ± 0.3b	3.0 ± 0.3b	3.1 ± 0.2b	2.4 ± 0.2b	2.5 ± 0.2b
	100,000	3.5 ± 0.3b	3.5 ± 0.2b	3.0 ± 0.3b	3.3 ± 0.3b	2.6 ± 0.3b	2.5 ± 0.2b
	200,000	3.7 ± 0.2b	3.8 ± 0.1b	3.4 ± 0.2b	3.1 ± 0.3b	3.2 ± 0.1c	2.7 ± 0.2bc
Feed conversion	Control	1.888		2.163		2.433	
	50,000	3.074	3.409	4.125	3.598	2.995	2.814
	100,000	5.517	4.108	6.453	3.943	4.244	3.047
	200,000	5.262	8.242	10.161	6.959	14.468	5.611

[1]Within each parameter of each assay, values followed by a common letter are not significantly different ($P \leq 0.05$).
[2]Each value is the mean of 10 replicates.

if this was true, the oocyst stock solutions collected by both techniques were periodically assayed. Cleaning and concentration of *E. maxima* oocysts resulted in insufficient numbers of sporulated oocysts for assaying.

The efficacy of the bicarbonate-ether and sedimentation techniques for maintaining storage stability are shown in Tables 2-4.

TABLE 4.—*Initial and three-month assays of* Eimeria tenella *(trial 2) comparing efficacy of bicarbonate-ether and sedimentation methods of cleaning oocysts*[1]

Parameter	Dose	Initial assay		Three-month assay	
		Bicarbonate-ether	Sedimentation	Bicarbonate-ether	Sedimentation
Mortality (%)	Control	0		0	
	50,000	30	40	30	10
	100,000	40	50	30	20
	200,000	40	20	40	60
Weight gain (gm.)[2]	Control	72.2 ± 5.7a		94.7 ± 3.4a	
	50,000	18.4 ± 6.1b	22.1 ± 7.4b	39.6 ± 8.6c	62.7 ± 11.6b
	100,000	10.0 ± 3.3b	14.7 ± 4.9b	9.8 ± 4.2d	27.4 ± 11.7cd
	200,000	3.9 ± 1.3b	5.5 ± 1.8b	−39.0 ± 8.3e	4.9 ± 4.4d
Lesion score[2]	Control	0.0a		0.0a	
	50,000	3.3 ± 0.2b	3.2 ± 0.3b	2.9 ± 0.3bc	2.4 ± 0.3b
	100,000	3.6 ± 0.2b	3.5 ± 0.2b	3.2 ± 0.3cd	3.4 ± 0.2cd
	200,000	3.7 ± 0.2b	3.3 ± 0.2b	3.5 ±0.2cd	3.7 ± 0.2d
Feed conversion	Control	2.346		2.392	
	50,000	6.505	5.855	4.343	3.327
	100,000	11.290	7.891	14.327	5.832
	200,000	27.795	16.055	—[3]	28.367

[1]Within each parameter of each assay, values followed by a common letter are not significantly different ($P \leq 0.05$).
[2]Each value is the mean of 10 replicates.
[3]Feed conversion could not be calculated due to the average weight gain of the group being a negative number.

Of the four parameters measured, statistical analysis of weight gain permitted the best standard for comparison. Although there were numerical differences between weight gains within a dose level, bicarbonate-ether had no more a deleterious effect on initial oocyst viability than did water. In the three and six month assays, a trend developed: the weight gain (within a dose level) resulting from bicarbonate-ether-cleaned oocysts was always numerically lower than weight gain resulting from oocysts cleaned by sedimentation. Also, in some of these assays the lowered weight gain was statistically significant. This was seen in the six month assay of *E. tenella*, trial 1, (Table 3) for doses of 100,000 and 200,000. This was reinforced for three month assays in *E. tenella*, trial 1, at the 200,000 dose level and *E. mivati* at the 100,000 dose level.

The ability to inflict mortality (Tables 2-4) diminished as oocyst suspensions cleaned by either technique were stored. Feed conversions (Tables 2-4) inversely paralleled weight gain. As weight gain decreased with increasing dose levels, feed conversions increased. Variability in lesions produced within treatment groups (Tables 2-4) ruled out usefulness of this parameter in judging storage stability of oocysts from the two techniques.

The ability of the oocysts cleaned by the two techniques to produce infection changed as they were stored. The main effect of the two concentration techniques on weight gain exhibited a change from no significance in all the initial assays to significance as the stock solutions were stored and periodically assayed.

The quantity of fecal suspension cleaned by the bicarbonate-ether technique depends on the size and quantity of centrifuged tubes used. In our study 50 ml. tubes were used to promote ease in handling. The time required to clean the aliquot after it was removed from the fecal suspension was never more than 30 minutes. This time includes the centrifugations, resuspensions and cleaning with ether.

In summary, the advantages of the bicarbonate-ether method are:

1) Large amounts of fecal material may be cleaned in a short period of time,
2) The purity of the cleaned suspension of oocysts is such as to yield high rates of sporulation efficiency, with possibly a maximum in total oocyst recovery, and
3) A prolonged storage stability of sporulated oocysts to affect coccidiosis.

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NEWS AND NOTES

(Continued from page 2065)

he started his own business.

In 1973 he began a Master of Science program in the Department of Animal and Poultry Science, completing it in 1974. He is working for a Ph.D. degree in the Department, majoring in applied animal behaviour. His primary interest is feeding behaviour in poultry, seeking to improve feed utilization by accenting behavioural, psychological and social factors. He has also received an Ontario Graduate Scholarship.

EUROPEAN POULTRY CONFERENCE

The 5th European Poultry Conference will be held in Malta, September 5 to 11, 1976.

The Scientific Programme will consist of six morning sessions and four afternoon sessions. The morning sessions will consist of three papers from invited speakers, and six or more contributed papers. The first part will occupy 1-1/2 hours, the second part 2 hours. The four afternoon sessions will consist of symposia on special topics.

1. Themes and subjects for the six technical sessions, chosen in consultation with the Working Groups of the European Federation of the W.P.S.A. are: Nutrition—saving of costs and nutrients in poultry feeding.

(Continued on page 2090)

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L2	1	S	GREEN, JACKIE/AU
L3	1	S	URAIZEE, FAROOQ/AU
L4	0	S	BULL, LANCE/AU
L5	7780	S	COCCIDIOSIS
L6	98	S	OOCYTS
L7	3	S	(ALKALI METAL DICHROMATE)
L8	13390	S	EIMERIA
L9	0	S	L8 AND L7
L10	9365	S	OOCYSTS
L11	3204	S	L10 AND L8
L12	0	S	L11 AND ALKALI METAL DICHROMATE
L13	35	S	L11 AND (10,000)
L14	29		DUP REM L13 (6 DUPLICATES REMOVED)
L15	4335	S	(SODIUM HYPOCHLORITE OR CLOROX)
L16	0	S	L14 AND L15

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FILE 'BIOSIS, SCISEARCH, VETU, VETB, AGRICOLA' ENTERED AT 17:14:44 ON 08 OCT 2003

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L1          9365 S OOCYSTS
L2          4260 S (SODIUM HYPOCHLORITE)
L3         19875 S SPORULATION
L4            10 S L2 AND L3
L5             8 DUP REM L4 (2 DUPLICATES REMOVED)
L6          2213 S ISOSPORA
L7             7 S L6 AND L2
L8            94 S CYSTOISOSPORA
L9             1 S L8 AND L2
L10            3 S CRYPTOSPRIDIUM
L11         9417 S CRYPTOSPORIDIUM
L12           34 S L11 AND L2
L13           22 DUP REM L12 (12 DUPLICATES REMOVED)
L14           43 S L1 AND L2
L15            0 S L14 AND (10,000)
L16           30 DUP REM L14 (13 DUPLICATES REMOVED)

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